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ORIGINAL RESEARCH

Synthesis and biological evaluation of some stilbene derivatives

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Abstract Several *trans* and *cis* stilbenes with substitution on the olefinic bridge were synthesized and characterized by IR, NMR and mass spectroscopy in an effort to obtain substances that could be more readily formulated. All the synthesized compounds were screened against Molt4/C8, CEM and L1210 cell lines. None of these compounds were endowed with pronounced cytostatic activity. However, Schiff derivatives emerged as cytostatic agents (IC_{50}: 0.77–10 μg/ml) that deserve further investigation.

Keywords Stilbenes · Cytostatic · Schiff · Olefine

Introduction

Cancer is one of the main reasons of death in both men and women. One promising natural product is resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) (RSV), a phytoalexin found in grapes and in red wine. The interest in this molecule has considerably increased in the last 10–12 years. The anticancer activity of resveratrol was first revealed by its ability to reduce the incidence of carcinogen-induced development of cancers in experimental animals (Jang *et al*., 1997; Dong, 2003). Since then, it has been demonstrated that it possesses chemopreventive and cytostatic properties via the inhibition of tumor initiation, promotion and progression (Jang *et al*., 1997). It has also been demonstrated that RSV inhibits ribonucleotide reductase catalyzing the rate limiting step of de novo DNA synthesis (Fontecave, 1998). It exerts a nonselective cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibition (Jang and Pezzuto, 1999). The antioxidant properties of resveratrol have been related to its polyphenolic nature, especially to the presence of hydroxyl groups (Wright *et al*., 2001; Stivala *et al*., 2001). Phenolic groups are also known as an important structural determinant for estrogen receptor (ER) binding (Sadler *et al*., 1998; Ekena *et al*., 1998; Gao *et al*., 1999). Various *trans* stilbene compounds were reported to be inhibitors of Cytochrome P450 (CYP). Resveratrol showed an inhibitory effect on human CYP1A1 and CYP1B1 (Chun *et al*., 1999; Chang *et al*., 2000). Recently, rhapontigenin, a natural hydroxystilbene, showed a strong selectivity of CYP1A1 inhibition (Chun *et al*., 2001), and it was found that the selectivities and inhibitory potency of stilbene compounds tested against CYP1s were sensitive to the substitution patterns on the *trans* stilbene template. Cushman *et al.* (1992) have synthesized and evaluated analogues of (*Z*)-l-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as potential cytotoxic and antimitotic agents. Lion *et al.* (2005) synthesized a novel family of monohydroxylated (*E*)-stilbenes and studied their ability to inhibit the growth and induce apoptosis in human tumor cell lines. Heynekamp *et al.* (2006) synthesized various substituted *trans* stilbene including analogue of resveratrol and proved that they inhibit the human tumor necrosis factor α-induced activation of transcription factor nuclear factor kappaB. Sangjun *et al*. (2009) synthesized *cis* stilbene derivative related to VIOXX and studied for their inhibitory effects on cell cycle progression and anti-estrogenicity in human adenoma breast cancer MCF-7 cells.

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Moran *et al*. (2009) synthesized fluorinated analogues of resveratrol and assayed on a variety of cell lines, primarily the non-small lung carcinoma cell line DLKP-A.

In this article we performed isosteric modifications of resveratrol by keeping the stilbene backbone of RSV to design substances that could be more readily formulated by (i) replacing the hydroxyl by various functional groups and (ii) introducing substituents on olefinic carbons and (iii) Schiff bases derivatives.

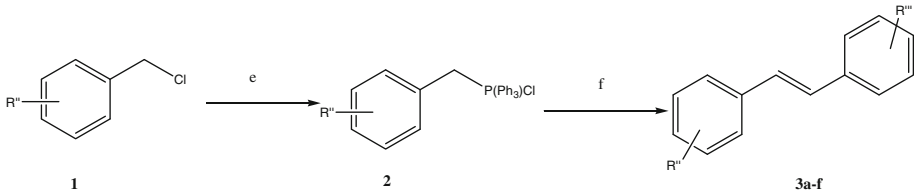
Results and discussion

Chemistry

Wittig reaction was carried out by using phosphonium chloride (**2**) with aryl aldehyde in benzene in the presence of sodium hydride. Excess of sodium hydride was quenched by addition of methanol and then chloroform and water were added. After evaporating, organic layer and residue were purified by preparative TLC using 5% ethanol in hexane as the eluent (**3a–f**) (Scheme 1, Table 1).

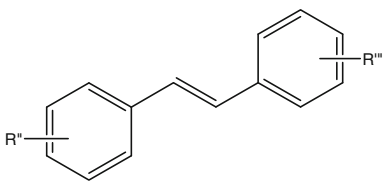
Several derivatives containing acidic and basic functional groups were prepared in an attempt to make compounds that were more soluble in water and therefore be formulated more easily (Cushman *et al*., 1992). Base-catalyzed condition of phenyl acetic acid (**4**) with aryl aldehyde (**5**) in the presence of triethylamine gave the carboxylic acid (**6a–c**) (Scheme 2, Table 2). Reaction of $SOCl_2$ with the carboxylic acid (**6**) in refluxing benzene gave the corresponding acid chlorides (**7**), which, upon subsequent reaction with appropriate amines, gave compounds (**8a–g**) (Scheme 2, Table 2). **9a** and **b** derivatives were prepared by compound **6** with methanol using catalytic H_2SO_4.

Several Schiff derivatives (**12a–d**) were prepared as bioisosteric compounds of stilbene by refluxing aldehyde (**10**) and aromatic amines (**11**) in toluene in a Dean stark apparatus (Scheme 3, Table 3).



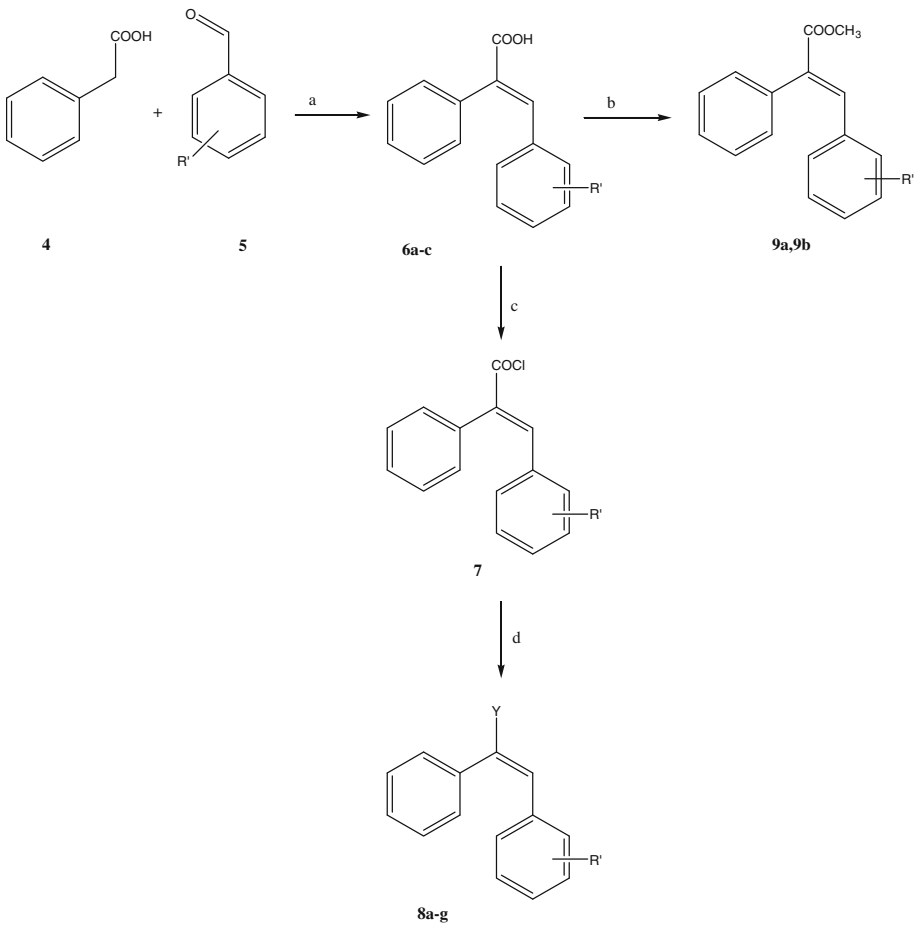
Scheme 1 General procedure for the preparation of **3a–f** reagents and conditions: *e* CH_3CN, triphenyl phosphine, *f* NaH, aryl aldehyde, benzene, 0–5°C

Table 1 *Trans* stilbenes **3a–f**, and standard melphalan



S. no	R″	R‴	MP (°C)	IC_{50} (μg/ml)		
				L1210	Molt4/C8	CEM
3a	4-F	4-OCH_3	136–138	26 ± 10	>200	156 ± 75
3b	4-CH_3	4-$N(CH_3)_2$	139–140	143 ± 4.0	>200	>200
3c	4-CH_3	4-OCH_3	143–145	>200	>200	>200
3d	4-F	2-F	115–121	62 ± 15	61 ± 9.0	83 ± 11
3e	4-CH_3	2-F	135–138	72 ± 1.0	30 ± 7.0	78 ± 18
3f	4-F	H	121–123	>200	>200	>200
Melphalan	–	–	–	2.1 ± 0.02	3.2 ± 0.6	2.5 ± 0.2

IC_{50}: 50% inhibitory concentration



Scheme 2 General procedure for the preparation of **6a–c**, **8a–g**, **9a** and **b** reagents and conditions: *a* triethyl amine, acetic anhydride, dil HCl *b* H_2SO_4, MeOH *c* $SOCl_2$, benzene, *d* Amines, 2 h, rt

Biological evaluation

The effects of 18 stilbene analogues and 4 analogues of Schiff derivatives as bioisosteres of stilbene on cell growth are summarized in Tables 1, 2 and 3. This group of compounds include 6 *trans* stilbenes (**3a–f**) (Table 1), 12 *cis* stilbene derivatives with substitution on the bridge connecting two phenyl rings (**6a–c**, **8a–g**, **9a and b**) (Table 2), and bioisosteres of stilbenes as Schiff derivatives (**12a–f**) (Table 3).

All the *trans* stilbenes (**3a–f**) were poorly cytostatic (Table 1). The IC_{50} values ranged between 26 and >200 μM.

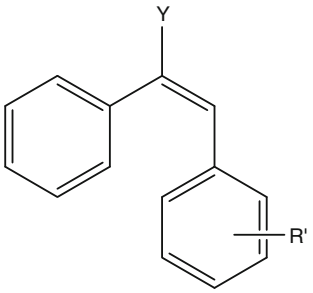
Regarding the *cis* stilbenes with substitutions on the olefinic bridge (Table 2), there was not much improvement in cytostatic activity. In separate experiments, a COOH group was introduced on the olefinic carbon linkage, and this resulted in the formation of compounds **6a**, **b** and **c** (IC_{50} 37 to >200 μg/ml). However, when the –COOH group of the compound was converted to a methyl ester (compound **9a** and **b**) or the *N*-diethylamide (**8c**), the cytostatic activity somewhat increased, as particularly noted for **8c** and **9a**. However, the *N*-methylamide derivatives (**8a**, **b**, **f** and **g**) were clearly less cytostatic.

In the third set of modifications, we designed Schiff derivatives as a bioisosteric replacement of *trans* stilbenes. All these compounds (**12a–d**) turned out to be much more potent than *trans* stilbenes and *cis* restricted stilbenes in inhibiting tumor cell proliferation (IC_{50}: 0.77–10 μg/ml). Several cytostatic activities were as potent as those found for melphalan. These data justify more in-depth studies on this type of compounds by introducing a variety of modifications on the aryl moieties of these molecules.

Conclusion

Several *trans* and *cis* stilbenes with substitution on the olefinic bridge were synthesized in an effort to obtain substances that could be more readily formulated. None of these compounds emerged as cytostatic compounds. But

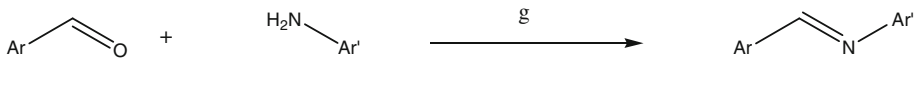
Table 2 Compounds **6a–c**, **8a–g** and **9a** and **b**



S. no	Y	R′	MP (°C)	IC_{50} (μg/ml)		
				L1210	Molt4/C8	CEM
6a	COOH	2-Cl	105–108	76 ± 12	37 ± 24	93 ± 10
6b	COOH	2-F	170–172	>200	89 ± 2.0	>200
6c	COOH	2-OH	139–140	>200	>200	>200
8a	$CONHCH_3$	2-Cl	100–103	65 ± 25	77 ± 0.0	60 ± 1
8b	$CONHCH_3$	4-OCH_3	182–185	68 ± 47	166 ± 16	128 ± 58
8c	$CON(C_2H_5)_2$	2-Cl	60–63	24 ± 11	14 ± 2	18 ± 12
8d	$CONHC_2H_5$	H	99–102	132 ± 40	112 ± 44	26 ± 8
8e	$CONHC_2H_5$	4-OCH_3	135–138	148 ± 45	150 ± 71	116 ± 87
8f	$CONHCH_3$	2-F	77–80	85 ± 9	72 ± 3	89 ± 15
8g	$CONHCH_3$	2-OH	69–70	>200	>200	>200
9a	$COOCH_3$	2-Cl	113–115	25 ± 10	16 ± 1	19 ± 7
9b	$COOCH_3$	4-OCH_3	127–130	66 ± 46	71 ± 9	71 ± 46

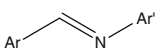
IC_{50}: 50% inhibitory concentration

Scheme 3 General procedure for the preparation of **12a–d** reagents and conditions: *g* toluene, Dean Stark apparatus



10 **11** **12a-d**

Table 3 Compounds **12a–d**



S. no	Ar	Ar′	MP (°C)	IC_{50} (μg/ml)		
				L1210	Molt4/C8	CEM
12a	2-Cl-phenyl	3-Cl-4-F-phenyl	57–55	6.4 ± 2.9	3.9 ± 3.0	10 ± 4
12b	2-Cl-phenyl	4-F-phenyl	35–37	6.0 ± 0.7	0.77 ± 0.22	2.1 ± 1.7
12c	2-Cl-phenyl	4-Br-phenyl	38–40	2.1 ± 1.4	1.5 ± 0.9	4.5 ± 2.6
12d	2-thiophene	3-Cl-4-F-phenyl	40–42	3.2 ± 1.8	16 ± 8	25 ± 18

IC_{50}: 50% inhibitory concentration

Schiff derivatives emerged as interesting antiproliferative compounds against the three tumor cell lines evaluated.

Experimental

Chemistry

The solvents (AR grades) were obtained from Sd Fine Chem., Mumbai, and E. Merck, Mumbai. The reagents (puriss grade) were obtained from Fluka and E. Merck. FT-IR spectra were recorded in KBr powder on a Jasco V410 FT-IR spectrometer by diffuse reflectance technique. $^{1}H/^{13}C$ NMR spectra were measured in $CDCl_3$ and DMSO-d6 on a Bruker Ultraspec AMX 400 MHz spectrometer. The reported chemical shifts were against that of TMS. Elemental Analysis was carried out at CDRI, Lucknow on Elementar Vario EL III, Carlo Erba 1108.

*General procedure for the preparation of stilbenes **3a–f***

A stirred solution of benzyl chloride **1** (2.88 g, 31.7 mmol) in acetonitrile (20 ml) was treated with triphenylphosphine (8.57 g, 32.7 mmol), and the mixture was refluxed with stirring for 12 h and then evaporated. The crude product was purified by crystallization from chloroform and ether, affording 95% yield as a white solid **2**. Sodium hydride (72 mg, 3 mmol) was added in portions to a well-stirred suspension of phosphonium chloride **2** (2 mmol) and aryl aldehyde (2 mmol) in benzene (20 ml) at 0–5°C, and the mixture was allowed to warm up to room temperature. After an additional stirring for 16 h, excess sodium hydride was quenched by the addition of methanol (1 ml), after which 30 ml of chloroform and water was added. The organic and aqueous layers were then separated. Distilled of the organic layer, the residue was purified by preparative TLC using 5% ethanol in hexane as the eluent.

*(E)-1-(4-Fluorophenyl)-2-(4-methoxyphenyl)-ethene (**3a**)*

Yield 25%; FT-IR ν_{max} cm^{-1} (KBr): 3042–3017, 2924–2844, 1604, 1573, 1511 and 1463. Anal. Calcd for $C_{15}H_{13}OF$: C, 78.93; H, 5.74. Found: C, 78.25; H, 5.55.

*(E)-1-(4-Methylphenyl)-2-(4-dimethylaminophenyl)-ethene (**3b**)*

Yield 23%; Anal. Calcd for $C_{17}H_{19}N$: C, 86.03; H, 8.07; N, 5.90 Found: C, 86.25; H, 7.89; N, 6.05. FT-IR ν_{max} cm^{-1} (KBr): 3197–3021, 2916–2808, 1600, 1528, 1437 and 1363. ^{1}H-NMR ($CDCl_3$) δ; 7.42 (4H, m), 7.13 (2H, d, $J = 8$ Hz), 6.99 (1H, d), 6.92 (1H, d), 6.71 (2H, d, $J = 8.8$ Hz), 2.98 (6H, s, $-N(CH_3)_2$), 2.35 (3H, s, CH_3).

*(E)-1-(4-Methylphenyl)-2-(4-methoxyphenyl)-ethene (**3c**)*

Yield 25%; Anal. Calcd for $C_{16}H_{16}O$: C, 85.68; H, 7.19. Found: C, 85.34; H, 6.99. FT-IR ν_{max} cm^{-1} (KBr): 3049–3021, 2954–2916, 1603, 1515, 1439 and 1377. ^{1}H-NMR ($CDCl_3$) δ; 7.44 (2H, d, $J = 8.8$ Hz), 7.38 (2H, d, $J = 8$ Hz), 7.15 (2H, d, $J = 8$ Hz), 7.01 (1H, d, $J = 16.4$ Hz), 6.93 (1H, d, $J = 16.4$ Hz), 6.89 (2H, d, $J = 8.8$ Hz), 3.83 (3H, s, OCH_3), 2.36 (3H, s, $-CH_3$).

*(E)-1-(4-Fluorophenyl)-2-(2-fluorophenyl)-ethene (**3d**)*

Yield 26%; Anal. Calcd for $C_{14}H_{10}F_2$: C, 77.77; H, 4.66. Found: C, 77.34; H, 4.29. FT-IR ν_{max} cm^{-1} (KBr): 3077–3055, 2918, 1590, 1484 and 1438.

*(E)-1-(4-Methylphenyl)-2-(2-fluorophenyl)-ethene (**3e**)*

Yield 24%; Anal. Calcd for $C_{15}H_{13}F$: C, 84.88; H, 6.17. Found: C, 84.64; H, 5.99. FT-IR ν_{max} cm^{-1} (KBr): 3081–3056, 2953–2919, 1590, 1483 and 1437.

*(E)-1-(4-Fluorophenyl)-2-phenyl-ethene (**3f**)*

Yield 22%; Anal. Calcd for $C_{14}H_{14}F$: C, 84.82; H, 5.59. Found: C, 84.67; H, 5.25. FT-IR ν_{max} cm^{-1} (KBr): 3080–3023, 2953–2849, 1593, 1508 and 1448.

*General procedure for the preparation of compounds (**6a–c**)*

A mixture of phenyl acetic acid **4** (2 mmol) substituted benzaldehyde **5** (2 mmol) and triethylamine (0.5 ml) in acetic anhydride (5 ml) was heated under reflux for 12 h and poured into hot saturated sodium carbonate solution (50 ml) and left overnight. The mixture was extracted with ether (2 × 50 ml), the ether extracts were discarded, and the aqueous solution was acidified with dilute HCl, after which the precipitated product was filtered and dried. Recrystallization from ethyl acetate–hexane gave the pure products.

*(E)-3-(2-Chlorophenyl)-2-phenyl-prop-2-enoic acid (**6a**)*

Yield 33%; Anal. Calcd for $C_{15}H_{11}O_2Cl$: C, 69.64; H, 4.29. Found: C, 69.25; H, 4.15. FT-IR ν_{max} cm^{-1} (KBr): 3250–2500, 1685, 1425 and 1266. ^{1}H-NMR ($CDCl_3$) δ; 8.20 (1H, s), 7.37 (1H, d, $J = 8$ Hz), 7.31–7.26 (3H, m), 7.21–7.19 (2H, m), 7.15 (t, $J = 15.2$ Hz), 6.90 (1H, t,

$J = 15.2$ Hz), 6.76 (1H, d, $J = 8$ Hz). MS: *m/z* (%) 259 (M + 1).

*(E)-3-(2-Fluorophenyl)-2-phenyl-prop-2-enoic acid (**6b**)*

Yield 44%; Anal. Calcd for $C_{15}H_{11}O_2F$: C, 74.37; H, 4.58. Found: C, 74.09; H, 4.41. FT-IR ν_{max} cm^{-1} (KBr): 3200–2600, 1682 and 1620. ^{1}H-NMR ($CDCl_3$) δ; 8.15 (1H, s), 7.38–7.30 (3H, m), 7.25–7.21 (3H, m), 7.06–7.01 (1H, m), 6.81–6.74 (2H, m). MS: *m/z* (%) 243 (M + 2).

*(E)-3-(2-Hydroxyphenyl)-2-phenyl-prop-2-enoic acid (**6c**)*

Yield 46%; Anal. Calcd for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03. Found: C, 75.12; H, 4.79. FT-IR ν_{max} cm^{-1} (KBr): 3405, 3109–3051, 2970, 1713, 1613, 1454 and 1262.

*General procedure for the preparation of compounds (**8a–g**)*

A mixture of carboxylic acid **6** (0.5 mmol) and thionyl chloride (1 ml) and benzene (10 ml) was refluxed for 6 h. The excess thionyl chloride and benzene were removed at reduced pressure, and the residue **7** was subsequently mixed with amines (5 ml) and kept at room temperature for 2 h. The precipitated product was filtered, washed subsequently with 2% NaOH solution and water and dried. The product was purified with suitable solvent.

*(E)-N-Methyl-3-(2-chlorophenyl)-2-(phenyl)-prop-2-enamide (**8a**)*

Yield 46%; Anal. Calcd for $C_{16}H_{14}ONCl$: C, 70.72; H, 5.19; N, 5.15. Found: C, 70.15; H, 5.07; N, 5.21. FT-IR ν_{max} cm^{-1} (KBr): 3320, 3078–3023, 2940–2931, 1654, 1617, 1403 and 1269. ^{1}H-NMR ($CDCl_3$) δ; 8.01 (1H, s), 7.35–7.33 (3H, m), 7.26 (2H, s), 7.20–7.18 (2H, m), 7.10–7.06 (1H, m), 6.85 (1H, t, $J = 18.8$ Hz), 6.65 (1H, d, $J = 7.6$ Hz), 5.55 (1H, s, br, NH, D_2O exchangeable), 2.88 (3H, s, $-CH_3$). MS: *m/z* (%) 272 (M + 1).

*(E)-N-Methyl-3-(4-methoxyphenyl)-2-(phenyl)-prop-2-enamide (**8b**)*

Yield 48%; Anal. Calcd for $C_{17}H_{17}NO_2$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.19; H, 6.25; N, 5.29. FT-IR ν_{max} cm^{-1} (KBr): 3442, 3099–3006, 2958–2836, 1661, 1599, 1514, 1460 and 1405. ^{1}H-NMR ($CDCl_3$) δ; 7.82 (1H, s), 7.48–7.42 (3H, m), 7.26–7.24 (2H, m), 6.90 (2H, d, $J = 8.8$ Hz), 6.64 (2H, d, $J = 8.8$ Hz), 5.44 (1H, s, br, NH, D_2O exchangeable), 3.74 (3H, s, OCH_3), 2.84 (3H, s, CH_3).

*(E)-N-Diethyl-3-(2-chlorophenyl)-2-(phenyl)-prop-2-enamide (**8c**)*

Yield 23%; Anal. Calcd for $C_{19}H_{20}NOCl$: C, 72.72; H, 6.42; N, 4.46. Found: C, 72.02; H, 6.99; N, 4.51. FT-IR ν_{max} cm^{-1} (KBr): 3442, 3077–3020, 2986–2873, 1621, 1573, 1470 and 1433. ^{1}H-NMR ($CDCl_3$) δ; 7.39 (1H, d, $J = 8$ Hz), 7.25–7.21 (5H, m), 7.16–7.11 (1H, m), 6.98–6.95 (1H, m), 6.79 (1H, s), 3.48 (2H, d, $J = 8$ Hz), 3.36 (2H, d, $J = 8$ Hz), 1.18 (3H, t, $J = 12$ Hz), 1.02 (3H, t, $J = 12$ Hz).

*(E)-N-Ethyl-3-(phenyl)-2-(phenyl)-prop-2-enamide (**8d**)*

Yield 44%; Anal. Calcd for $C_{17}H_{16}NOCl$: C, 71.45; H, 5.64; N, 4.90. Found: C, 69.98; H, 5.25; N, 5.01. FT-IR ν_{max} cm^{-1} (KBr): 3329, 3077–3018, 2974–2871, 1654, 1528, 1493 and 1261.

*(E)-N-Ethyl-3-(4-methoxyphenyl)-2-(phenyl)-prop-2-enamide (**8e**)*

Yield 44%; Anal. Calcd for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.81; N, 4.98. Found: C, 76.02; H, 6.53; N, 5.01. FT-IR ν_{max} cm^{-1} (KBr): 3303, 3053–3004, 2966–2842, 1650, 1606, 1525, 1459 and 1349. ^{1}H-NMR ($CDCl_3$) δ; 7.81 (1H, s), 7.49–7.43 (3H, m), 7.27–7.25 (2H, m), 6.90 (2H, d, $J = 8.8$ Hz), 6.65 (2H, d, $J = 8.8$ Hz), 5.41 (1H, s, br, NH, D_2O exchangeable), 3.74 (3H, s, OCH_3), 3.37–3.30 (2H, q, CH_2), 1.07 (3H, t, $J = 14.5$ Hz, CH_3).

*(E)-N-Methyl-3-(2-fluorophenyl)-2-(phenyl)-prop-2-enamide (**8f**)*

Yield 45%; Anal. Calcd for $C_{16}H_{14}NOF$: C, 75.28; H, 5.53; N, 5.49. Found: C, 69.89; H, 5.36; N, 5.35. FT-IR ν_{max} cm^{-1} (KBr): 3316, 3079–3022, 2933, 1656, 1619, 1454 and 1271. ^{1}H-NMR ($CDCl_3$): δ; 8.01 (1H, s), 7.43–7.39 (3H, m), 7.24–7.22 (2H, m), 7.17–7.15 (1H, m), 7.01–6.97 (1H, m), 6.74 (1H, t, $J = 15.2$ Hz), 6.61 (1H, t, $J = 15.2$ Hz), 5.53 (1H, s, br, NH, D_2O exchangeable), 2.86 (3H, s, $-CH_3$).

*(E)-N-Methyl-3-(2-hydroxyphenyl)-2-(phenyl)-prop-2-enamide (**8g**)*

Yield 38%; Anal. Calcd for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.19; H, 5.55; N, 5.61. FT-IR ν_{max} cm^{-1} (KBr): 3406, 3106–3057, 2966–2929, 1714, 1667, 1612, 1488 and 1454. ^{1}H-NMR (CDCl3) δ; 8.01 (1H, s), 7.35–7.33 (3H, m), 7.26 (2H, s), 7.20–7.18 (2H, m), 7.10–7.06 (1H, m), 6.85 (1H, t, $J = 18.8$ Hz), 6.65 (1H, d,

$J = 7.6$ Hz), 5.55 (1H, s, br, NH, D_2O exchangeable), 2.88 (3H, s, $–CH_3$).

*General procedure for the preparation of compounds **9a** and **b***

Concentrated H_2SO_4 (0.5 ml) was added to a stirred solution of carboxylic acid (0.5 mmol) in absolute methanol (20 ml) and the mixture was heated under reflux for 6 h. Excess of methanol was removed by evaporation, and the residue was poured into ice water. The product was extracted with ether (2 × 20 ml), and the combined extracts were washed with 2% aqueous NaOH solution (2 × 50 ml) followed by water (200 ml). Evaporation of ether layer gave the desired product.

*(E)-Methyl-3-(2-chlorophenyl)-2-(phenyl)-prop-2-enoate (**9a**)*

Yield 48%; Anal. Calcd for $C_{16}H_{13}O_2Cl$: C, 70.46; H, 4.80. Found: C, 69.99; H, 4.98. FT-IR ν_{max} cm^{-1} (KBr): 3057, 2991–2848, 1704, 1570, 1496 and 1387.

*(E)-Methyl-3-(4-methoxyphenyl)-2-(phenyl)-prop-2-enoate (**9b**)*

Yield 45%; Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.10; H, 6.10. Found: C, 75.98; H, 5.79. FT-IR ν_{max} cm^{-1} (KBr): 3055, 2954–2837, 1704, 1597, 1513 and 1385.

*General procedure for the preparation of Schiff derivatives (**12a–d**)*

A solution of aldehyde **10** (1 mmol) and aniline **11** (1 mmol) in toluene (5 ml) were heated to reflux in a Dean Stark apparatus for 16 h. After the solvent was removed in vacuo, the crude product was recrystallised from ethanol to give 45–50% yield.

*(E)-3-Chloro-N-(2-chlorobenzyledene)-4-fluoro benzenamine (**12a**)*

Yield 45%; Anal. Calcd for $C_{13}H_8Cl_2N$: C, 58.24; H, 3.01; N, 5.22. Found: C, 58.01; H, 2.99; N, 5.31. IR (KBr) cm^{-1}: 3089–2996, 2922, 1620 and 1494. ^{1}H-NMR ($CDCl_3$): $\delta = 8.89$ (1H, s), 8.20 (1H, d, $J = 8$ Hz), 7.46–7.43 (2H, m), 7.41–7.36 (1H, m), 7.33–7.31 (1H, m), 7.21–7.12 (2H, m). MS: *m/z* (%): 268 (M).

*(E)-N-(2-Chlorobenzyledene)-4-fluorobenzenamine (**12b**)*

Yield 51%; Anal. Calcd for $C_{13}H_9ClN$: C, 66.82; H, 3.88; N, 5.99. Found: C, 66.79; H, 3.58; N, 6.31. FT-IR ν_{max} cm^{-1} (KBr): 3075–2997, 2918–2848, 1617 and 1498. ^{1}H-NMR ($CDCl_3$): $\delta = 8.9$ (1H, s), 8.22 (1H, d, $J = 7.6$ Hz), 7.47–7.35 (3H, m), 7.27–7.23 (2H, m), 7.13–7.08 (2H, m), 6.85 (1H, t, $J = 18.8$ Hz), 6.65 (1H, d, $J = 7.6$ Hz), 5.55 (1H, s, br, NH), 2.88 (3H, s, $–CH_3$).

*(E)-N-(2-Chlorobenzyledene)-4-bromobenzenamine (**12c**)*

Yield 52%; Anal. Calcd for $C_{13}H_9ClNBr$: C, 53.01; H, 3.08; N, 4.75. Found: C, 52.75; H, 2.94; N, 4.94. FT-IR ν_{max} cm^{-1} (KBr): 3061–3018, 2918–2848, 1618 and 1484. ^{1}H-NMR (CDCl3): $\delta = 8.92–8.90$ (1H, m), 7.26–7.22 (1H, m), 7.56–7, 52 (2H, m), 7.47–7.38 (2H, m), 7.26 (1H, d, $J = 9.6$ Hz), 7.16–7.12 (2H, m).

*(E)-3-Chloro-4-fluoro-N-(thiophen-2-ylmethylene)-benzenamine (**12d**)*

Yield 56%; Anal. Calcd for $C_{11}H_7ClFNS$: C, 55.12; H, 2.94; N, 5.84. Found: C, 55.01; H, 3.01; N, 5.55. FT-IR ν_{max} cm^{-1} (KBr): 3060, 2954, 2886, 1615 and 1492. ^{1}H-NMR (CDCl3): $\delta = 8.53$ (1H, s), 7.54 (1H, d, $J = 5.2$ Hz), 7.50 (1H, d, $J = 3.6$ Hz), 7.29–7.26 (1H, m), 7.17–7.08 (3H, m).

Cytostatic activity

The methodology for measuring the cytostatic activity in Molt 4/C8, CEM and L1210 assays has been published previously (Baraldi *et al.*, 2004). In brief, varying concentrations of compounds were incubated at 37°C with the cells for 72 (human Molt 4/C8 or CEM T-lymphocytes) or 48 h (murine L1210 cells). After the incubation period, the cell number was counted by a coulter counter (Harpenden Herz, UK).

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